Institute Report No. 307

Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN) in Guinea Pigs

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DIVISION OF TOXICOLOGY

October 1988

Toxicology Series: 143

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN) in Guinea Pigs (Toxicology Series 143)--Blatt *et al.*

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Edwin S. Beatrice 26 Oct 88

Edwin S. Beatrice (date)
COL, MC
Commanding

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION

1b. RESTRICTIVE MARKINGS

2a. SECURITY CLASSIFICATION AUTHORITY

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; distribution is unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
Institute Report No. 307

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Mammalian Toxicology Branch
Division of Toxicology

6b. OFFICE SYMBOL (If applicable)
SGRD-ULE-T

7a. NAME OF MONITORING ORGANIZATION
US Army Biomedical Research and Development Laboratory

6c. ADDRESS (City, State, and ZIP Code)
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

7b. ADDRESS (City, State, and ZIP Code)
Fort Detrick
Frederick, MD 21701-5010

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
US Army Medical Research & Development Command

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Fort Detrick
Frederick, MD 21701-5012

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
627204	835	AB	DA 303913

11. TITLE (Include Security Classification)
Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN) in Guinea Pigs)

12. PERSONAL AUTHOR(S)
Gerald F. S. Hiatt, John R. G. Ryabik, and Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 3/7/85 TO 4/15/85	October 1988	27

16. SUPPLEMENTARY NOTATION
Toxicology Series 143

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Dermal sensitization,
Diethyleneglycol dinitrate, Buehler test
DEGDN, Guinea pigs

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Diethyleneglycol dinitrate (DEGDN) was tested for its potential to produce sensitization via contact with the skin. Testing was performed on male guinea pigs using the Buehler Dermal Sensitization method. No evidence of dermal sensitization to DEGDN was obtained in this study.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL
Edwin S. Beatrice, COL, MC

22b. TELEPHONE (Include Area Code)
415-561-3600

22c. OFFICE SYMBOL
SGRD-ULZ

DD Form 1473, JUN 86 Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

ABSTRACT

Diethyleneglycol dinitrate (DEGDN) was tested for its potential to produce sensitization via contact with the skin. Testing was performed on male guinea pigs using the Buehler Dermal Sensitization method. No evidence of dermal sensitization to DEGDN was obtained in this study.

Key Words: Dermal Sensitization, Diethyleneglycol Dinitrate, DEGDN, Buehler Test, Guinea Pigs

PREFACE

TYPE REPORT: Dermal Sensitization GLP Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 85005

STUDY DIRECTOR: Don W. Korte, Jr., PhD, MAJ, MSC

PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

CO-PRINCIPAL INVESTIGATOR: John R.G. Ryabik, SP4, BS

PATHOLOGIST: Paul W. Mellick, DVM, PhD, COL, VC
Diplomate American College of
Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Diethyleneglycol Dinitrate

INCLUSIVE STUDY DATES: 7 March - 15 April 1985

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of diethyleneglycol dinitrate in guinea pigs.

ACKNOWLEDGMENTS

SSG James D. Justus, BS, SP4 James J. Fischer, SP4 Scott L. Schwebe, Charlotte Speckman, and Richard A. Spieler provided animal care and facilities management. CPT Earl W. Morgan, DVM, assisted with the research. MAJ Larry D. Brown, DVM, provided research and administrative guidance as Director of the Propellants Toxicity Testing Project. Callie B. Crosby and Colleen S. Kamiyama provided administrative and clerical support during the study performance and report preparation.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85005 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte 13 OCT 88
DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

Paul W. Mellick
PAUL W. MELLICK, DVM, PhD / DATE
COL, VC
Pathologist

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Conrad Wheeler 23 Apr 86
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Analytical Chemist

John R.G. Ryabik 22 Apr 86
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Co-Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 17 October 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Statement of Compliance

1. This is to certify that the protocol for GLP Study 85005 was reviewed on 5 March 1985.

2. The institute report entitled "Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN)," Toxicology Series 143, was audited on 13 August 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Dermal Sensitization Potential of Diethyleneglycol Dinitrate (DEGDN) in Guinea Pigs -- Hiatt *et al*

INTRODUCTION

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to evaluate the dermal sensitization potential of diethyleneglycol dinitrate in guinea pigs.

MATERIALS

Test Substance

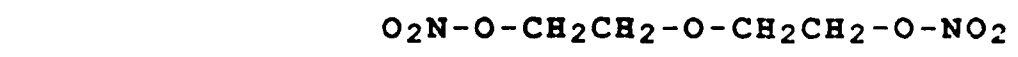
Chemical Name: Diethyleneglycol Dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

Code number: LAIR Code No. TA047

Physical State: Liquid

Molecular Structure:



Empirical formula: $C_4H_8N_2O_7$

Source: Hercules Incorporated
Radford Army Ammunition Plant
Radford, VA

Other test substance information is presented in Appendix A.

Vehicle for Test Substance

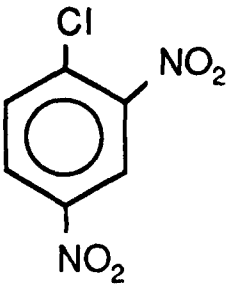
DEGDN is a liquid and produced no acute dermal irritation when applied neat in a pilot study. For the present study, DEGDN was therefore applied at a 100 % concentration using no vehicle.

Positive Control

Chemical Name: Dinitrochlorobenzene (DNCB)

Chemical Abstract Service Registry No.: 97-00-7

Molecular Structure:



Empirical Formula: $C_6H_3N_2O_4Cl$

Vehicle for Positive Control

The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Certified Laboratories, Inc. (Philadelphia, PA). Isotonic (0.9%) saline was obtained from Travenol Laboratories, Inc. (Deerfield, IL). The expiration date for this lot (7C95OXO) was October 1985. Additional positive control substance information is presented in Appendix A.

Animal Data

Thirty-two male guinea pigs, Hartley strain (Charles River Breeding Laboratories, Wilmington, MA) were used for this study. They were identified individually with ear tags numbered 85E0102 to 85E0133, inclusive. Two animals were selected for quality control necropsy evaluation on receipt at LAIR (animals arrived under GLP study 84046). Animal weights on transfer to this study ranged from 336 to 481 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow® Diet 5026 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 22.2°C to 27.8°C and relative humidity in a range of 32 to 50%. The photoperiod was 12 h of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-82 "Buehler Dermal Sensitization Test" (2) and Environmental Protection Agency (EPA) guidelines (3).

Acclimation and Group Assignment

The guinea pigs were quarantined for 19 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Ten animals were randomly assigned to each of three groups based on their tag numbers.

Dosage Levels

Diethyleneglycol dinitrate was applied neat, as a 100% concentration. A pilot study, using extra animals from a previous study, indicated the 100% solution to be non-irritating under the conditions of this test. Since no vehicle was needed to dilute the diethyleneglycol dinitrate a vehicle control group was not used in the study.

A sensitization control group was included in the study. Dinitrochlorobenzene, a known potent sensitizing agent (4), was applied to this group of ten animals, at a 0.1%

concentration, as the positive control. In addition, a negative control group received diethyleneglycol dinitrate only on the day of challenge dosing.

Compound Preparation

Diethyleneglycol dinitrate was applied neat and therefore required no preparation. A dinitrochlorobenzene (DNCB) positive control dosing solution was prepared by first adding 30 mg DNCB to 1 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to the skin to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures (5- 7) utilized in this study were developed by Buehler and Griffith to closely resemble the human repeated insult patch test (8). Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization a negative control group was added that received only the challenge dose.

During the induction phase, the experimental and positive control groups were dosed with 0.5 ml of the appropriate solution applied topically under a one-inch square gauze patch. This procedure was performed for three consecutive weeks (12, 19 and 26 Mar 85). On the day before each dosing an approximately 3-inch (7.6 cm) square area on the left flank of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp, Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp, Stamford, CT). The patch was taped with Blenderm® hypoallergenic surgical tape (3M Corp, St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vetrap® (3M Corp, St. Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was marked off for scoring.

Animals were challenged two weeks (9 Apr 85) following the third induction dose. The experimental group and the positive control group received two 0.5-ml doses, one applied to the old site on the left flank and the other to a new site on the right flank. The negative control group received only a single 0.5-ml dose, which was applied to the left side. The procedures for clipping, shaving, wrapping, and the exposure period remained the same.

In Buehler's procedure (5-7), skin reactions are scored only at 24 and 48 h and only after the challenge dose. In the present study, skin reactions were scored 24, 48, and 72 h after each induction dose, as well as after the challenge dose. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema), and 3 (marked erythema). The results are expressed both in terms of incidence (the number of animals showing responses of 1 or greater at 24, 48, or 72 h) and severity (the sum of the test scores divided by the number of animals tested). Results from the left flank are compared with those from the right flank and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-h exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose is applied. For consistency with induction procedures, this step was replaced by clipping and shaving a 3-inch (7.6 cm) square area on the left flank of the animals the day before dosing.

A historical listing of study events appears in Appendix C.

Deviations from Study Protocol

The DNCB solution was maintained at approximately 65°C before dosing the guinea pigs. This was necessary to keep the DNCB in solution, but did not result in thermal insult to the animals' skin as the aliquot for dosing cooled quickly during pipetting and application to the patch. Significant sensitization was produced by DNCB with this method.

RESULTS

Table 1 summarizes the incidence of reactions 24, 48, and 72 h after each dose. There were no reactions observed in response to diethyleneglycol dinitrate, at any time in the study.

This lack of response to diethyleneglycol dinitrate is reflected in Table 2, which reports the severity of skin reactions at 24, 48, and 72 h. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. Since diethyleneglycol dinitrate produced no reaction whatsoever, the severity index was also 0.0 at all scoring times.

Dinitrochlorobenzene (DNCB) produced a marked response at all time points after the second and third induction doses, as well as after the challenge dose. Between 90% and 100% of the DNCB-treated animals exhibited a response 24 h following these induction and challenge doses. These reactions persisted, yielding scorable effects in 50-70% of the animals at 72 h after dosing.

Beginning with the second induction, severity scores for these responses to DNCB ranged from 0.6 to 1.4 at the 24 h scoring period (Table 2). The highest score, 1.4, was observed on the left (induction) flank in response to the challenge dose. By 48 h the reactions had subsided somewhat and the severity scores ranging from 0.6 to 1.1 reflected this decrease. A further reduction was evident by 72 h, with the severity index ranging between 0.5 and 0.8.

No responses whatsoever were observed in the negative control (challenge dose of diethyleneglycol dinitrate only) group.

The individual 24-h, 48-h, and 72-h scores for all animals appear, by group, in Appendix D.

No lesions were found at necropsy that could be attributed to the test compound. The veterinary pathologist's report appears in Appendix E.

TABLE 1

Incidences of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
			24 Hours		
DEGDN	0/10	0/10	0/10	0/10	0/10
Negative Control*	----	----	----	0/10	----
DNCB	0/10	9/10	10/10	10/10	5/10
			48 Hours		
DEGDN	0/10	0/10	0/10	0/10	0/10
Negative Control*	----	----	----	0/10	----
DNCB	0/10	8/10	8/10	10/10	5/10
			72 Hours		
DEGDN	0/10	0/10	0/10	0/10	0/10
Negative Control*	----	----	----	0/10	----
DNCB	0/10	5/10	5/10	5/10	7/10

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 2

Severity† of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
			24 Hours		
DEGDN	0.0	0.0	0.0	0.0	0.0
Negative Control	---	---	---	0.0	---
DNCB	0.0	0.9	1.3	1.4	0.6
			48 Hours		
DEGDN	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	---
DNCB	0.0	0.8	0.8	1.1	0.6
			72 Hours		
DEGDN	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	---
DNCB	0.0	0.5	0.5	0.8	0.8

†Severity scale: 0=no reaction, 1=slight erythema, 2=moderate eythema, and 3=marked erythema.

*The Negative Control Group received only a challenge dose of the test compound.

DISCUSSION

Dermal Irritation and Sensitization

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference between the two is the mechanism responsible for this inflammation.

Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis.

Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical. Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. During the induction phase (3 weeks in the present study) a clone of T lymphocytes proliferates which is sensitized specifically to the eliciting antigen. Upon subsequent exposure to the antigen, these T lymphocytes release mediators, i.e., lymphokines, that initiate and amplify an inflammatory reaction at the site of contact.

Although both types of reactions can appear grossly similar in experimental animals and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, while sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, while only a fraction of the population normally becomes sensitized to a given chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction (a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, while the reaction to a sensitizer increases over two to four exposures after the

initial contact. An irritant produces inflammation of rapid onset with short duration while a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to an irritant may spread beyond the area of contact, whereas sensitization reactions are usually circumscribed.

The features of irritation and sensitization were used by Buehler and Griffith (5-7) to establish guidelines for differentiating between the two. In evaluating a dermal sensitization study they recommend comparing the results from a challenge dose in the experimental group with those for the negative control group:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- often fade within 24 to 48 h, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, the intensity/severity of inflammation being greater at 72 to 96 h than at 24 to 48 h.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy is evaluated by the method of Draize *et al* (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory response is graded. Evaluation of sensitization potential is accomplished by repeated application, at lower nonirritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow the immune system to elaborate and increase its specific reactivity to the chemical. A challenge dose is then given, and the resulting inflammatory reaction is graded. Analysis of the incidence, severity, and timing of the reaction to the challenge dose gives an estimate of the sensitizing potential of the study compound.

Diethyleneglycol Dinitrate

Diethyleneglycol dinitrate was evaluated for its ability to elicit a delayed-hypersensitivity reaction via dermal contact. As tested using the method of Buehler and Griffith (5-7), diethyleneglycol dinitrate produced no response indicative of either dermal sensitization or irritation. Therefore in this study diethyleneglycol dinitrate showed no evidence of potential to elicit an immunologic response.

Sensitization produced by diethyleneglycol dinitrate would have been detected by this study. A hypersensitivity-type response, characteristic of that observed previously within the Institute (10), was reliably elicited by DNCB in the present group of animals. Although DNCB is capable of producing primary irritation, the characteristics of responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also the response to DNCB was observed only after two or more exposures and the severity generally increased with the number of previous exposures.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than humans, this negative test result does not guarantee that diethyleneglycol dinitrate will not sensitize humans. It does indicate that diethyleneglycol dinitrate is unlikely to sensitize humans and that its potential is low enough to permit testing in humans.

CONCLUSION

Diethyleneglycol dinitrate exhibited no potential for inducing dermal sensitization under conditions of this study.

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Appendix A: CHEMICAL DATA

Chemical name: Ethanol, 2,2'-oxybisdinitrate

Alternate chemical name: Diethyleneglycol dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

LAIR Code No.: TP047

Chemical structure:

$$O_2N\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}NO_2$$

Molecular formula: $C_4H_8N_2O_7$

Molecular weight: 196

Physical state: Pale yellow liquid

Density (g/cm^3): 1.38[1]

Analytical data: Refer to the attached data sheet, ARRCOM Form 213R. The compound chromatographed as a single peak (retention time 5.4 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% acetonitrile; flow rate, 0.9 ml/min; detection wavelength, 205 nm.[2] NMR (300 MHz, CD_3CN): 3.75 δ (complex multiplet, 4H, $-CH_2-O-CH_2-$), 4.61 complex

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[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland; US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA127846, p. 17.

[2] Wheeler CR. Toxicity Testing of Propellants. Laboratory Notebook #85-12-023, p. 31, Presidio of San Francisco, CA: Letterman Army Institute of Research.

Appendix A (cont.): CHEMICAL DATA

multiplet, 4H,-CH_2ONO_2).[3] Additional singlet signals of approximately equal intensity were observed at 2.08 d, and were due to sample impurities. Integration of all signals in the spectrum demonstrated that the sample contained 96.6% DEGDN. The impurities were not identified. IR(KBr): 2896, 1632, 1429, 1390, 1373,1279, 1139, 1032, 909, 857, 758, 707, 655, 572cm^{-1}.[4]

Stability: The DEGDN was shipped containing 18% acetone (a desensitizer) and arrived at LAIR on 12 December 1984. The acetone was removed by rotary evaporation prior to studies with the propellant. Analysis of the compound one year after it was received gave the results described above. Stability of the compound in corn oil (the dosing vehicle) was examined. As determined by HPLC, the concentration of DEGDN in corn oil emulsions 24 h after preparation was within 1% of the target value.[5]

Source: Radford Army Ammunition Plant, Radford, Virginia (prime contractor: Hercules Inc., Wilmington, Delaware).

Lot No.: RAD84M001S214

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[3] Ibid. pp. 44-48.

[4] Ibid. pp. 49-50.

[5] Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 57-60, Presidio of San Francisco, CA: Letterman Army Institute of Research.

Appendix A (cont): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC

REPORTS CONTROL SYMBOL: EXEMPT-Para 7-2a AR 335-15 | PAGE 1 OF 1

TO: | FROM: | DATE: December 5, 1984 | MATERIAL: Diethylene Glycol Dinitrate (DEGDN)

MANUFACTURER: HERCULES INCORPORATED
RADFORD ARMY AMMUNITION PLANT

CONTRACT NO.: DAAA09-77-C-4007

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
RAD84M001S214	-	1	5 lbs

PLACE MANUFACTURED: RADFORD ARMY AMMUNITION PLANT, RADFORD, VIRGINIA

SPECIFICATION AND AMENDMENT/DRAWING NO.: DOD-D-64015

SECTION B - DESCRIPTION OF MATERIAL

Requirements	Limit	Results
82.2°C Potassium Iodide Starch Paper Heat Test (KI)	10 minutes minimum	12
Nitrogen, %	14.10 minimum	14.15
Water, %	Info Only	0.43
Acidity	None	None
Alkalinity	None	None

REMARKS: DEGDN is desensitized with 15% or more of acetone for a total weight of 5 lbs, and packed in a DOT 6D 5 gallon drum with a DOT 2S liner, overpacked in a DOT-6J 30 gallon capacity drum with vermiculite as a cushioning agent around the 5 gallon drum and contained in the 30 gallon drum. Requested by shipping Order AMCCOM and COR letter SMCRA dated November 28, 1984 (DOT Exemption 5704).

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: HERCULES INCORPORATED

TESTING CONDUCTED BY: HERCULES INCORPORATED

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

12-5-84 (DATE) — F.A. WALKER (SIGNATURE)

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

Dec 6, 1984 (DATE) — (TITLE) — FOR THE COMMANDER (SIGNATURE)

ARRCOM Form 213-R, 10 Aug 77 SEQUENCE No. 374

Appendix A (cont.): CHEMICAL DATA

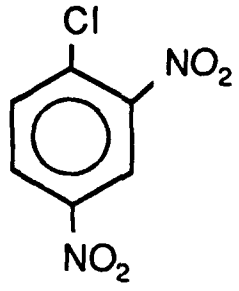
POSITIVE CONTROL

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52-54° C[1]

Purity:

The compound was designated as 95% pure by source.

Analytical Data:

Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.[2] Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (d).[3] Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).[4]

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[1]Windholz M, ed. The Merck Index. 10th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

[2]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 9-10, Presidio of San Francisco, CA: Letterman Army Institute of Research.

[3]*Ibid.* pp. 11-12.

[4]*Ibid.* pp. 13-16.

Appendix A (cont.): CHEMICAL DATA

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm^{-1}. The IR spectrum was very close to the Sadtler reference spectrum.[5] Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR ($CDCl_3$): d 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J_{ortho} = 8.7 Hz, J_{meta} = 3.6 Hz), 8.74 (1 H, d, J_{meta} = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.[6] GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses (m/z) of 202 and 204 confirmed the identity of the major peak as DNCB.[7]

Lot Number: 11F-0543

Source: Sigma Chemical Co.
St. Louis, MO

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[5]Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

[6]Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

[7]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 13-15, Presidio of San Francisco, CA: Letterman Army Institute of Research.

Appendix B: ANIMAL DATA

Species: *Cavia porcellus*

Strain: Hartley

Source: Charles River Breeding Laboratories
Wilmington, MA

Sex: Male

Date of birth: 2 February 1985

Method of randomization: By assigned animal number IAW LAIR SOP OP-ISG-21.

Animals in each group: 10 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tag, tag numbers 85E0102 to 85E0133 inclusive.

Pretest conditioning: Quarantine/acclimation
20 Feb - 11 Mar 1985

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
7 Mar 85	Animals were received from GLP study 84046.
11 Mar 85	Animals were weighed and randomized into groups.
11 Mar 85	All animals, except negative control group, were clipped and shaved.
12 Mar 85	Test animals, except negative control group, were given first induction dose.
13 Mar 85	Test animals, except negative control, were scored for 24-h skin reaction.
14 Mar 85	Test animals, except negative control group, were scored for 48-h reaction.
15 Mar 85	All animals, except negative control group, were scored for 72-h skin reaction.
18 Mar 85	All animals, except negative control group, were clipped and shaved. All animals were weighed.
19 Mar 85	All animals, except negative control group, were given induction dose.
20 Mar 85	All animals, except negative control group, were scored for 24-h skin reaction.
21 Mar 85	All animals, except negative control group, were scored for 48-h skin reaction.
22 Mar 85	All animals, except negative control group, were scored for 72-h skin reaction.
25 Mar 85	All animals, except negative control group, were clipped and shaved. All animals were weighed.
26 Mar 85	All animals, except negative control group, were given induction dose.
27 Mar 85	All animals, except negative control group, were scored for 24-h skin reaction.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

Date	Event
28 Mar 85	All animals, except negative control group, were scored for 48-h skin reaction.
29 Mar 85	All animals, except negative control group, were scored for 72-h skin reaction.
1 Apr 85	All animals were weighed.
8 Apr 85	All animals were weighed, clipped, and shaved.
9 Apr 85	All animals were given the challenge dose.
10 Apr 85	All animals were scored for 24-h skin reaction.
11 Apr 85	All animals were scored for 48-h skin reaction.
12 Apr 85	All animals were scored for 72-hour skin reaction.
15 Apr 85	All animals were weighed and delivered to the Necropsy Suite for sacrifice and gross necropsy.

APPENDIX D: Individual Dermal Scores

GROUP: Positive Control COMPOUND: DNCB	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE					
										LEFT FLANK			RIGHT FLANK		
ANIMAL NUMBER	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
85E0105	0	0	0	1	0	0	2	1	0	1	1	2	0	0	0
85E0106	0	0	0	1	1	0	2	1	1	2	1	2	1	1	1
85E0107	0	0	0	1	1	1	1	1	0	1	1	1	0	0	0
85E0108	0	0	0	1	1	1	1	1	1	2	2	2	1	1	1
85E0111	0	0	0	1	1	1	1	1	0	1	1	0	1	1	1
85E0118	0	0	0	1	1	0	1	1	1	1	1	0	0	0	1
85E0125	0	0	0	1	1	0	2	1	1	1	1	0	0	1	1
85E0126	0	0	0	1	1	1	1	1	1	2	1	1	2	2	2
85E0130	0	0	0	0	0	0	1	0	0	2	1	0	1	0	1
85E0133	0	0	0	1	1	1	1	0	0	1	1	0	0	0	0

APPENDIX D (cont.): Individual Dermal Scores

GROUP: TEST COMPOUND: DEGDN	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE					
										LEFT FLANK			RIGHT FLANK		
ANIMAL NUMBER	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
85E0104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX D (cont.): Individual Dermal Scores

GROUP: Negative Control COMPOUND: DEGDN	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE					
										LEFT FLANK			RIGHT FLANK		
ANIMAL NUMBER	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
85E0109	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0112	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0113	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0115	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0119	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0120	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0122	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0124	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0127	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA
85E0132	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA

APPENDIX E: Pathology Report

LAIR Gross Pathology Report
GLP Study 85005

Study: GLP 85005, Toxicology Branch, Division of Comparative Medicine and Toxicology, LAIR

Test: Buehler Dermal Sensitization

Investigator: Dr. Gerald F.S. Hiatt

Test Substance: DEGDN (CAS No. 693-21-0)

Findings:

Group	Animals/Group	Lesions
Test Compound	10	0[1]
Positive Control	10 (DNCB)	5[2]
Negative Control	10	2[3]

1. No gross lesions were recognized in the organs or tissues of any of the 10 animals to which the test compound was administered.

2. Three of the 5 lesions were in animal #85E0125. These consisted of (1) distention of the left ureter (0.5 cm in diameter) filled with flocculent pale yellow fluid, (2) dilation of the left renal pelvis, and (3) yellow-green viscid material in the left vas deferens. These lesions were interpreted as a unilateral ascending genitourinary infection with obstruction and were unrelated to test material administration or the experimental procedure. One animal, 85E0105 had a 0.5 x 1.5 cm area of hair loss and dry, crusty red skin posterior to the last rib on the left side. The cause of this lesion may have been infection secondary to sensitization caused by DNCB. The fifth lesion in this group was a 2 mm focus of necrosis on the medial lobe of the liver. The lesion was considered unrelated to the procedure since such lesions are commonly encountered in guinea pigs.

3. The right eye of animal 85E0132 had a congenital dermoid cyst in the conjunctiva. The liver of animal 85E0112 contained seven pinpoint sized red and white foci in the liver, probably focal areas of necrosis. Neither of these lesions was test compound or procedure related.

PAUL W. MELLICK, DVM, PhD
COL VC
Veterinary Pathologist
3 February 1986

APPENDIX E (cont.): Pathology Report

Pathology Report
GLP Study 85005

Buehler Dermal Sensitization
DEGDN (CAS No. 693-21-0)

Dose Group: Test Compound 0.5 ml

LAIR Pathology Acession No.	Animal ID No.	Gross Findings
37355	85E0104	Not Remarkable (NR)
37341	85E0110	NR
37345	85E0114	NR
37347	85E0116	NR
37348	85E0117	NR
37352	85E0121	NR
37354	85E0123	NR
37359	85E0128	NR
37360	85E0129	NR
37362	85E0131	NR
	Dose Group: Positive Control (DNCB)	
37336	85E0105	0.5 x 1.0 cm area dry crusty skin
37337	85E0106	NR
37338	85E0107	NR
37339	85E0108	NR
37342	85E0111	NR
37349	85E0118	NR
37356	85E0125	(1) Distention, left ureter (2) Dilatation, left renal pelvis (3) Yellow-green material, left vas deferens
37357	85E0126	NR
37361	85E0130	NR
37364	85E0133	2 mm necrotic focus, liver.
	Dosage Group: Negative Control	
37340	85E0109	NR
37343	85E0112	7 pinpoint foci of necrosis, liver
37344	85E0113	NR
37346	85E0115	NR
37350	85E0119	NR
37351	85E0120	NR
37353	85E0122	NR
37355	85E0124	NR
37358	85E0127	NR
37363	85E0132	1 mm nodule with hair, conjunctiva right eye

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234